The Continuous Culture of Microorganisms:

Continuous Culture System

- *A microbial population of can be maintained in the exponential growth phase and at a constant biomass concentration for extended periods.*

- ***Open System**: system with constant environmental conditions maintained through continual provision of nutrients and removal of wastes .*

Two Common Major Types of Continuous Culture Systems:

(1). Chemostats

- *Sterile medium is fed into the culture vessel at the same rate as the media containing the Mos is removed.*

(2). Turbidostats

- *Photocell that measures the absorbance or turbidity of the culture in the growth vessel.*

Environmental Factors on Growth:

The growth of Mos are effected by Chemical and Physical surroundings:

- *Procaryotes are present anywhere life can exist.*
- ***Extremophiles**: Mos grow in harsh environments*
  Live 1.5 miles below the earth's surface, w/o oxygen, and below 60 °C.

Temperature

- *Microbial cell temperature directly reflects that of the cell's surrounding.*

- *Most bacteria can grow over a* **temperature range** *of about 30˚ or more but have a narrow range for* **optimal growth**.

- As we *decrease the temperature below the optimum, we see a decline in growth rate that is consistent with enzymatic activity, but then it becomes very steep, giving rise to a fairly well defined minimal growth temperature.*

- *Above the optimum temperature, we see the growth rate decline very steeply, which gives rise to a sharply defined maximum growth temperature*

It is not known what sets the upper and lower temperature although they are thought to

- *reflect properties of the membrane lipids,*
- *effects on protein conformation, and/or initiation of protein synthesis.*

Temperature Sensitivity of Enzyme-Catalyzed Reactions:

- *A temp rise, increases the growth rate due to the velocity of an enzyme-catalyzed reaction.*
- *Velocity will double for every 10º C rise in temperature.*
- *As rate increase, the metabolism is more active at higher temp, Mo grow faster.*
- *Example: 10 -- 30, Velocity is 15 What is the velocity of the cell at 50º C?*

High Temperatures:

- *Damage MOs by denaturing enzymes, transport carriers, and other proteins*
- *Membranes are disrupted, lipid bilayer simply melts and disintergrates.*

Low Temperatures:

- *membranes solidify and enzymes don't work properly.*

The temperature range of an organism can be used as a classifying characteristic. All bacteria have distinct cardinal temperatures:

Cardinal Temperatures Growth Temperatures:

- *Minimum*
- *Optimum*
- *Maximum*

Pc can grow at much higher Temp than EC

***Psychrophiles** can grow at temperatures between 0-20º, optima growth is 15º*

- *Frequently found in naturally cold waters and soils.*
  Such as the Artic and Antarctic.
- *Examples include the Pseudomonads and Bacillus,*
- *Enzymes, transport systems and protein synthetic mechanisms function well as low temp.*

- *Cell membrane have high levels of unsaturated fatty acid and remain semifluid when cold.*

Psychrotrophs or Facultative Psychrophiles *can grow at 0 to 7 º C.*

- *Optima 20-30 º C*
- *Maxima 35 º C*
- *Psychrotrophic bacteria and fungi are important in spoilage of refrigerated foods*

Most bacteria are ***Mesophiles*** *and grow between 20-45 ºC.*

- *Those that are found in the mammalian body have an optimum temperature of 37-44 º C, Maxima is 45 º C.*
- *Those found in the environment have an optimum of about 30 degrees C*
- *Almost all human pathogens are mesophiles, env. is around 37ºC.*

Thermophiles *grow at temperature of 55º C or higher.*
Minimum of 45 ºC and optima between 55 and 65 º C.

- *Majority of prokaryotes*
- *Flourish in composts, self-heating hay stacks, hot water lines, and hot springs.*
- *More heat stable enzymes and protein synthesis systems / funct at higher temp.*
- *Membranes lipids more saturated and have higher melting points causing membrane to remain in tact at higher temp.*
- *These organisms are extremely useful in that they serve as sources for exceptionally stable forms of enzymes (i.e. bacillus stearothermophilus)*

Hyperthermophiles *are thermophiles that can grow at 90º C or above,*

- *Procayotes gowth optima between 80 and 113 º C.*
- *Do not grow well below 55 º C.*
- These organisms are extremely useful in that they serve as sources for exceptionally stable forms of enzymes (i.e. bacillus stearothermophilus)

pH <u>(Acidity or alkalinity)</u>

pH is a measure of the Hydrogen Ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration (expressed in terms of molarity).

pH scale 0.0 (1.0 M H^+) – 14.0 (1.0 x 10^{-14}M H^+)

Each unit represents a tenfold change in hydrogen ion concentration.

- **Bacteria can also be classified by the pH ranges in which they grow**.
- The internal pH of the cell remains close to neutral,
- An organism's tolerance to fluctuations in pH reflects the capacity of the membrane pumps to maintain that pH

Acidophiles *grow best below pH 4.0*.

- The vinegar forming acetobacter and some of the sulfur oxidizing bacteria can tolerate the pH values as low as ~0 (the pH of 1N sulfuric acid)
- Growth range between 0 – 5.5

Neutrophiles

- Growth range is 5.5 to 8.0
- **Most bacteria and protozoa**

Alkalophiles (most grow best above ~pH 10)

- Growth range 8.5 to 11.5
- a few of the urea splitters, Alcaligenes faecalis, and vibrio cholerae can **thrive at pH levels as high as 9 and can tolerate levels greater than 10**.
- E. coli cannot withstand pH conditions greater than 8 or below 4.5.

a. $H_2O \leftrightarrow H^+ + OH^-$

$[H^+] = [OH^-] = 1.00 \times 10^{-7}$ mole per liter in pure water.

b. $pH = \log 1/[H^+]$

The pH of pure water is $\log (1)/(1.00 \times 10^{-7})$

$= \log (1.00 \times 10^{7}) = 7.00.$

c. Acids are pH 0 - 7, bases are pH 7 - 14.

d. Every increase of 1 pH unit is a 10-fold decrease in $[H^+]$.

Most bacteria grow between pH 5 and pH 8 (across a 1,000-fold difference in external hydrogen ion concentration).

A few extreme acidophiles (oddly, all are eukaryotes and archaea) can grow at or near pH 0.

Oxygen Concentration

Oxygen effects on growth

a. O_2 reacts with certain enzymes in the cell to form hydrogen peroxide (H_2O_2) and superoxide (O_2^-). These compounds can damage biological macromolecules.

b. Detoxifying enzymes: catalase, peroxidase, and superoxide dismutase.

c. Singlet oxygen (esp. produced in photosynthesis) can be quenched by carotenoids

d. Categories of microorganisms (by oxygen environment):

(1) Strict aerobes:
- Organism grow in the presence of atmospheric O_2

(2) Strict anaerobes
- Do not tolerate O2 at all and die in the presence of it.

(3) Facultative anaerobes
- Do not require O2 for growth but do grow better in its presence.

(4) Aerotolerant anaerobes
- Grow equally well whether it is present or not

(5) Microaerophiles (low oxygen)
- damaged by the normal atmospheric level of O_2 (20%) and require O_2 levels below the range of 2 to 10% for growth.

Most organism are very sensitive to the salinity of the environment or ***osmotic pressure****.*

The rigid structure of the bacterial cell wall enables it to grow over a wide range of osmotic pressures.

Most bacteria grow in ranges between 0.85% NaCl (physiological saline) and 3.5% NaCl (seawater)

Some bacteria have adapted mechanisms which enable them to withstand extremes of osmotic pressure

Halophiles grow best in environments where the osmotic pressure ranges between 18-24% NaCl.

Salinity (osmotic balance):

- [hi H2O in/ low H2O out (hi salt) =>becomes dehydrated;
- low salt environment, H2O rushes in and cell lyses

- Many microbial environments are salty. For example, the oceans cover about 71% of the Earth's surface. Cells must maintain an osmotic balance with the environment while not allowing high salt concentrations to inhibit essential metabolic processes.

- Salt concentration in human blood is about 0.85% NaCl (physiological saline).

- Sea water contains about 3.5% salt (mostly NaCl). The Dead Sea has a salt concentration of about 24%.

- A saturated salt solution contains about 35% NaCl.

- Microorganisms that grow in extreme high-salt environments (156-30% NaCl; salt pans, evaporating pools, etc.) are called halophiles .

halophile vs. halotolerant

d. Compatible solutes are produced to increase solute levels within cell without inhibiting cellular processes allowing the

e. Bacteria in the genus Halobacterium grow best in environments where the NaCl concentration is above 18%. They actively pump Na^+ ions out of the cell and pump K^+ ions in to maintain their osmotic balance. Energy for pumping is generated by a unique light-dependent photosynthetic process that is not based on chlorophyll.

Growth of bacterial cell cultures (populations)

1. Doubling time or generation time: the time between one cell division and the next (the time it takes for the population of cells to double in size).

    a. Doubling time depends upon the species and the culture conditions.

    b. Optimal doubling times: *E. coli* (20 minutes) versus *Mycobacterium tuberculosis* (15-16 hours).

    c. Natural environments: *E. coli* in the human intestine (10 hours), *Pseudomonas aeruginosa* in soil (2-3 days).

How does a bacterial population grow?

a. c. Conclusion: the number of cells will double with each new generation (exponential growth).

d. Exponential growth equation

$$N_2 = N_1 2^n \qquad \text{equation (1)}$$

N_2 = the number of bacterial cells at time t_2

N_1 = the number of bacterial cells at time t_1

n = the number of doublings

e. The logarithmic form of the equation:

$$\log N_2 = \log N_1 + n \log 2 \qquad \text{equation (2)}$$

f. Definition of doubling time:

$$g = (t_2 - t_1) / n \qquad \text{equation (3)}$$

g. Substitute equation (3) into equation (2):

$$\log N_2 = \log N_1 + \log 2 \, (t_2 - t_1) / g \qquad \text{equation (4)}$$

h. Substitute log 2 = 0.30:

$$\log N_2 = \log N_1 + 0.30 \, (t_2 - t_1) / g \qquad \text{equation (5)}$$

i. Usually N_2, N_1, and $(t_2 - t_1)$ are measured experimentally and we solve equation (5) for g. However, we can solve the equation for any unknown if all of the other quantities are given.

j. Example: for a culture of *E. coli*: let g = 20 minutes, N_1 = 1 cell, and $(t_2 - t_1)$ = 2 days (2,880 minutes). What is N_2?

(log 1 = 0)

$\log N_2$ = (log 1)+0.30 (2,880 minutes) / (20 minutes)= 43.2

$N_2 = 1.6 \times 10^{43}$ cells

If each cell weighs 1×10^{-12} grams:

(1.6×10^{43} cells) (1×10^{-12} g / cell) = 1.6×10^{31} g

= approximately 2,700 times the total mass of the Earth (6×10^{27} grams)